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FILED: December 22, 2003
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REMARKS

The present response is intended to be fully responsive to all points of objection and/or rejection raised by the Examiner and is believed to place the application in condition for allowance. Favorable reconsideration and allowance of the application is respectfully requested.

Status of Claims

Claims 1-22 and 25-34 are pending in the application. Claims 1-20 have been withdrawn from consideration. Claims 21-22 and 25-34 have been rejected.

CLAIM REJECTIONS UNDER 35 U.S.C. § 103

In the Office Action, the Examiner rejected claims 21-22 and 25-34 under 35 U.S.C. § 103(a), as allegedly unpatentable over Castell (I) or (II) in view of Braun and Rolfe. The Examiner alleged that Castell (I) and (II) each discloses a culture medium substantially free of animal products and containing *C. difficile*, comprising a compound derived from a vegetable and an iron source; that Braun disclosed a culture medium containing *C. difficile*, comprising yeast extract and a compound derived from a vegetable, such as trypticase soy broth; and that Rolfe disclosed cultivation of *C. difficile* on a thioglycolate medium that contains sodium thioglycolate. Further, the Examiner alleged that it would have been obvious to a person of average skill in the art to modify the culture media of Castell (I) and (II) by adding a soy hydrolyzate as taught by Braun and yeast extract as taught by Braun and Rolfe as additional nutrient sources, or by adding sodium thioglycolate as disclosed by Rolfe for the expected benefit of providing a suitable nutrient medium for anaerobic cultivation of *C. difficile* obtained from clinical samples.

The Examiner admitted, however, that the claimed invention differs from Castell (I) and (II) in the use of a soy hydrolyzate as opposed to grass extract, and that yeast extract and/or thioglycolate are not disclosed in Castell (I) and (II).

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Applicants agree with the Examiner that (a) the claimed invention differs from Castell (I) and (II) as described hereinabove, and (b) yeast extract and/or thioglycolate are not disclosed in Castell (I) and (II), and respectfully disagree with the rejection. Regarding Rolfe, the Examiner's allegation of obviousness appears to be based on the allegation that the casein hydrolyzate contained in the media of Rolfe and the vegetable hydrolyzates contained in the media of the present invention "are substantially degraded to amino acids and peptides prior to addition to the culture medium...[that] are not clearly distinguishable" from one another.

Applicants respectfully disagree. Contrary to the Examiner's allegations, the Encyclopedia of Bioprocess Technology (1999), volume 3, shows that amino acid compositions of hydrolyzates reflect on the amino acid content of the individual protein that is hydrolyzed."

"The source protein and method and degree of hydrolysis determine the nutritional characteristics of a hydrolyzate... Enzymatic digests contain free amino acids as well as small peptides while maintaining the vitamin content of the original source material. Enzymatic casein digests, reflecting the amino acid composition of the starting material, are high in tryptophan and low in cystine" (Encyclopedia of Bioprocess Technology, page 1650, second column, a copy of which is attached hereto as Appendix A; emphasis added).

Accordingly, the amino acid content would vary significantly between casein hydrolyzates and vegetable hydrolyzates.

Further, as admitted by the Examiner and as shown in the above quotation from the Encyclopedia of Bioprocess Technology, protein hydrolyzates contain peptides as well as amino acids. A person skilled in the art would know that the nature and sequence of the peptides would vary significantly, depending on the source protein. Accordingly, the nature and sequence of peptides contained would vary significantly between casein hydrolyzates and vegetable hydrolyzates.

Thus, contrary to the Examiner's allegations, casein hydrolyzates and vegetable hydrolyzates are clearly distinguishable from one another in both their amino acid compositions and the nature and sequence of the peptides contained therein.

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Page 4Objective indicia of non-obviousness

Further, even if, for the sake of argument, a prima facie case of obviousness had been established regarding the combination of the alleged prior art references, Applicants respectfully assert that the Examiner has ignored relevant objective indicia of non-obviousness.

First of all, Applicants respectfully point out to the Examiner that compositions of the present invention exhibit unexpected properties; namely, (a) high levels of bacterial growth and toxin production in animal-free media (page 1, paragraph 0012); (b) far superior Toxin A production after 3 days with soy peptone-containing fermentation medium, compared with vegetable peptone and other non-animal sources (page 5, Table 6 and discussion immediately below the table); (c) superior Toxin A production after 5 days with soy peptone-containing seed media, compared with plant and wheat peptones (pages 6-7, Table 11 and discussion immediately below the table); (d) far superior Toxin A production after 5 days with soy peptone-containing seed media, compared with plant and wheat peptones (pages 8-9, Table 19 and discussion immediately below the table); and (e) ability to grow *C. difficile* and produce Toxin A using animal-product free medium at all four stages of growth; namely, working cell stock culture preparation medium, first stage seed medium, second stage seed medium, and fermentation medium (page 8, paragraph 0091).

Further, the subject specification presents additional unexpected results; namely, enhanced toxin production in the presence of iron powder (page 8, Tables 16 and 17 and discussion below each table), as recited in dependent claims 25, 30, and 34 of the subject application.

Applicants therefore respectfully request that the rejection be withdrawn.

Further, Rolfe and Braun teach away from the subject invention by their repeated use of animal product-containing media for *C. difficile* cultivation. For example, Braun utilized either brain heart infusion medium or trypticase soy agar, which contains a pancreatic digest

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of the milk protein casein, as shown in page 1824 of Microbiological Media, a copy of which is attached hereto as Exhibit B.

Rolfe utilized chopped meat-glucose, brain heart infusion media, proteose peptone, and thioglycolate media. Proteose peptone is an animal product, as shown in the Difco Manual:

"Extensive investigations were undertaken at Difco, using peptic digests of animal tissue prepared under varying digestion parameters (Difco Manual 10th Edition, a copy of which is attached hereto as Exhibit C, page 696, first column).

Thioglycolate media contains casitone, a pancreatic digest of the milk protein casein, as shown in page 952 of the Difco Manual:

"Thioglycollate media, formulated with Bacto Yeast Extract and Bacto Casitone or pancreatic digest of casein, support growth of a wide variety of fastidious organisms having a range of growth requirements" (Difco Manual, page 952; emphasis added)

Applicants therefore respectfully request that the rejection be withdrawn.

Further, in arriving at the culture media of the present invention, Applicants have proceeded contrary to accepted wisdom in the art. Prior to the teachings of the present invention, animal-product free media were believed to be inferior for cultivation of *C. difficile*, as shown by the repeated use of animal product-containing media for *C. difficile* cultivation in the literature, for example in the Braun and Rolfe references. Applicants' use of animal-product free media thus proceeded contrary to accepted wisdom in the art at the time of filing the subject application.

Applicants therefore respectfully request that the rejection be withdrawn.

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In view of the foregoing amendments and remarks, the pending claims are deemed to be allowable. Their favorable reconsideration and allowance is respectfully requested.

Should the Examiner have any question or comment as to the form, content or entry of this Amendment, the Examiner is requested to contact the undersigned at the telephone number below. Similarly, if there are any further issues yet to be resolved to advance the prosecution of this application to issue, the Examiner is requested to telephone the undersigned counsel.

Please charge any fees associated with this paper to deposit account No. 50-3355.

Respectfully submitted,



Mark S. Cohen
Attorney/Agent for Applicant(s)
Registration No. 42,425

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Pearl Cohen Zedek Latzer, LLP
1500 Broadway, 12th Floor
New York, New York 10036
Tel: (646) 878-0800
Fax: (646) 878-0801

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